Detection of an endangered mammalian species using environmental DNA analysis

Carl M. Halford[1,2], Karl J. Moreton-Jones[2], Dr David J. Hill[1] and Dr Wera M. Schmerer[1].
[1]Wolverhampton School of Science, University of Wolverhampton, UK.
[2]Crestwood Environmental Ltd, Wolverhampton, UK.

Introduction

The UK water vole (*Arvicola amphibius*) population has been in severe decline for the past 70 years (Strachan *et al.* 1993).
The accurate and inexpensive monitoring of a species' distribution and population size is imperative to the efforts to conserve the species. The current methods for conducting presence/absence surveys for water vole rely upon observation of field signs, such as the presence of latrines and burrows (Strachan *et al.* 2011). These methods can lead to inaccurate estimations of the presence/absence of the species. Therefore, there is a need for additional survey methods to increase the accuracy and reduce the costs of water vole surveys.
Environmental DNA (eDNA) is the genetic material distributed by an organism into the environment that it inhabits.
This genetic material can be utilised to determine the presence of a specific-species at the location of sampling, providing the opportunity to accurately map the distribution of a species with relatively low financial cost, time-input and taxonomic expertise required, compared to traditional survey methods.
In this study, a real-time PCR (qPCR) assay for the detection of water vole specific eDNA has been designed and tested. The results presented show that the assay successfully detects water vole DNA. This assay will now be further tested and optimised to devise a method of detecting the presence/likely absence of water voles at specific aquatic locations.

Materials and methods

Design of species specific water vole primers and dual-labelled hydrolysis probe
Species-specific primers for water vole were designed using the Cytochrome b (*Cyt b*) gene sequence, situated within the mitochondrial genome (figure 1), obtained from Genbank (https://www.ncbi.nlm.nih.gov/genbank/). The sequences were aligned using the ClustalW alignment tool (Thompson et al. 1994), available on the Mega7.0 sequence analysis software (Kumar et al. 2016) and a consensus sequence was generated.
The consensus sequence was aligned with the *Cyt b* gene sequences from 68 related and/or sympatric species. The forward primer was then manually designed within highly variable regions and checked for secondary structures using the Integrated DNA Technologies (IDT) OligoAnalyzer 3.1 online tool (Owczarzy et al. 2008). The Primer-Blast online tool (Ye et al. 2012) was then utilised to check the species-specificity of the forward primers and to design appropriate species-specific reverse primers.
3 primer pairs were selected for testing and then validated by PCR (figure 2).

DNA extraction
A modified version of Qiagen's DNeasy Blood and Tissue (Qiagen GmbH, Hilden, Germany) DNA extraction protocol was utilised to extract the DNA from water vole and non-target species hair samples. Briefly, 10 hairs were selected and added to a 2 ml tube. Buffer ATL + Proteinase K (180 µl and 20 µl, respectively) were added to each tube. The samples were then incubated at 55 °C for 24 hours. The samples were then each added to a DNeasy spin column and the protocol carried out following the manufacturer's instructions. The DNA was then stored at 4 °C until further analysis.

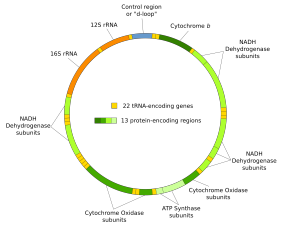
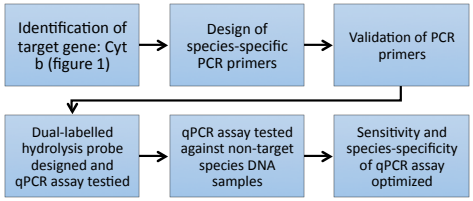


Figure 1. The mitochondrial genome, containing the Cytochrome b gene.

Real-time PCR assay parameters
The qPCR was performed in a total reaction volume of 25 µL, consisting of 3.0 µL DNA template, 12.5 µL TaqMan Environmental 2.0 master mix (ThermoFisher, Massachusetts, USA) 1.0 µL forward primer, 1.0 µL reverse primer and 1.0 µL dual-labelled hydrolysis probe. The qPCR was carried out in an Applied Biosystems 7500 Fast real-time PCR thermal cycler. 56 °C for 5 minutes and 95 °C for 10 minutes followed by 45 cycles of 95 °C for 30s and 62 °C for 30 s.
The limit of detection was measured by running a qPCR assay against a dilution series of water vole DNA, ranging from concentrations of 1.2 µg/mL to 0.012 pg/µL (figure 2).
The species-specificity of the qPCR assay was also tested against the DNA of non-target closely related and sympatric species.

Workflow of the development of a species-specific qPCR assay



Results

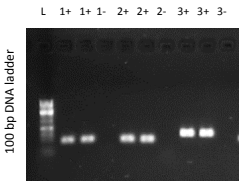


Figure 2: Polymerase chain reaction products amplified from water vole DNA

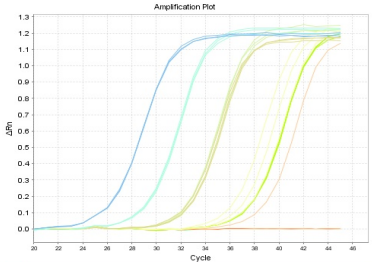


Figure 3. Preliminary dilution series.

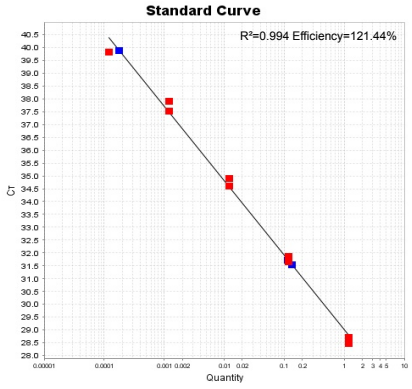


Figure 4. Standard curve

All 3 primer pairs tested amplified water vole DNA (figure 2)
Water vole DNA was successfully amplified by real-time PCR
The limit of detection for water vole DNA by qPCR was measured at a concentration of 1.2 pg/µl (figure 3)

The efficiency of the qPCR assay, by measurement of the slope of the standard curve, was calculated as 121.44% (figure 4)

No DNA from any tested closely related species was consistently amplified by the qPCR assay

Discussion

The qPCR assay developed in this study successfully amplifies water vole *cyt b* DNA allowing for the detection of DNA isolated from environmental water samples.

Further optimisation of the qPCR reaction conditions and mixture is required to maximise the sensitivity of the assay while preventing non-specific annealing to non-target DNA.

The qPCR assay will be tested against DNA samples extracted from water samples collected from habitats known to contain water vole populations in addition to habitats known to be free of water vole to confirm the accuracy and reliability of the assay.

The qPCR assay developed and tested in this study could provide an additional survey technique, to confirm the presence/likely absence of water vole at a particular aquatic site.

References

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